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Primary mediastinal clear cell lymphoma of B-cell type*

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Summary. This is a report on 8 mediastinal tumours that occurred in young adults (19–43 years, mean: 29.4); predominantly in females (6/8). Initial symptoms consisted of thoracic pain and venectasia and in only one case in B symptoms. After surgical tumour reduction, radiation and/or chemotherapy, local recurrence was observed in each case under clinical care; abdominal spread is presently suspected in 3 patients; 3 died 11, 13 and 22 months after diagnosis. None developed leukaemia. The tumours are B-cell neoplasms with a characteristic immunophenotype: leucocyte common antigen$^+$, common acute lymphoblastic leukaemia antigen$^-$, B 1-antigen$^+$, surface and cytoplasmic immunoglobulin$^-$. Flow cytometry revealed DNA-diploidy in 7 cases and a moderately (3.2–3.8%) to extremely high (8.0–20.6%) S-phase component. The proliferation associated antigen Ki67 was detectable in 10–60% of the tumour cell nuclei, thus stressing the considerable or rapid growth. Histopathology is characterized by a diffuse growth pattern and a clearness and abundance of cytoplasm of the pleomorphic tumour cells, which vary in size and nuclear morphology from patient to patient. Apoptoses are more numerous than mitoses. Fibrosis and focal necrosis are common, sclerosis is present in 3 cases. We suggest that primary mediastinal lymphoma of B cell type is a novel B-lymphoma variant.

Key words: Mediastinal lymphoma – Clear cell lymphoma – Immunoglobulin-deficient B cell lymphoma

Introduction

B-cell lymphomas can be diagnosed on a morphological basis using the Kiel classification (Lennert et al. 1978) and immunologically by their mono-

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clonal immunoglobulin (Ig) expression. However, it is well known that B-cell lymphomas tend to have defective immunophenotypes, such as a lack of heavy or light chains. Tumours producing only the J-chain have been reported (Mason and Stein 1981; Möller et al. 1982; Kelény 1985) and a complete loss of Ig and its constituents might also occur more frequently than initially assumed (Horning et al. 1984; Knowles II et al. 1985). Recently Gregg et al. (1984) published a series of Ig-negative follicular centre cell lymphomas, diagnosed as such by morphology. There are also B-cell tumours so deviant in morphology that they may mimic T-cell lymphomas (Mirchandani et al. 1985; Porfrey et al. 1985), Hodgkin's disease (Linch et al. 1985) and even carcinomas (Gatter et al. 1985). Against this background monoclonal antibodies reacting with B-cell restricted antigens can become extremely useful for the detection of B-cell tumours whose cells neither look like B cells nor express Ig. Using a set of monoclonal antibodies including anti-B1, whose antigen is known to be B-cell restricted and whose expression is functionally linked with B-cell activation and differentiation (Fedder et al. 1985), we were able to detect a number of B-cell lymphomas lacking both typical morphology and Ig-expression. Among them we found a conspicuously homogenous group of tumours having a considerable number of clinical, histological and immunohistological aspects in common (Eberlein-Gonska et al. 1985).

Material and methods

Tumour tissue from 8 patients with mediastinal tumour (Table 1) was transferred immediately after surgical removal (in a fresh and non fixed state) to our laboratory. Representative samples were snap frozen in liquid nitrogen (for immunohistology), in absolute ethanol (for cytophotometry) and in Bouin's fixative (for routine processing). Routine stains from the fixed and paraffin-embedded material comprised HE, PAS, Giemsa and the Gomori reticulin method modified by Pap (vd. Otto 1984). Looking at these sections a conventional and preliminary diagnosis was made (Table 1) and reported to the clinician.

DNA flow cytometry (FCM). The relative DNA content of nuclei was measured by means of flow cytometry. For this purpose single cell suspensions were prepared by mincing the material mechanically in an acid pepsin solution. After DNA-specific fluorochrome marking with 4'6-diamino2-phenylindole 10^5 cells were measured in the impulse cytophotometer ICP 22 (Phywe, Göttingen, FRG). The evaluation of the resulting distribution of the relative DNA content yielded, by means of a special computer programme (Haag 1980), the percentage of cells in $G_{0\,1}$-, S-, and G_2+M-phase. The resulting diagrams furnished information concerning the ploidy of the cells in $G_{0\,1}$-phase. The $(G_2+M)/S$-ratio and the proliferation index $((S+G_2+M)\times 100/(G_{0\,1}+S+G_2+M))$ were calculated instantly (for further details vd. Feichter et al. 1985).

Immunohistology

Tissue. 2 µm thick paraffin sections were deparaffinized, bleached in methanol/H_2O_2 for 20 min and exposed to Pronase (1 mg/ml PBS, pH 7.4) for 5 min at room temperature, prior to incubation with the first antiserum/antibody. 4–6 µm thick cryostat sections were made, air dried overnight at room temperature and subsequently fixed in acetone for 10 min at room temperature, prior to storing in −20° C or exposure to the first antibody.

Reagents. Unconjugated rabbit derived antisera to human μ-, γ-, α-, ε-, κ-, and λ-chains, and human epidermal keratin (diluted 1:50–1:100), swine anti-rabbit immunoglobulin serum

Table 1. Clinical data

Case no	Name	Sex	Age at diagn. (years)	First clinical symptoms	Primary localisation	Preliminary diagnosis[a]	Figure	Clinical course after surgical intervention, radiation and chemotherapy
1	BM	fem.	19	Left breast venectasia, facial oedema	(Para) Thymic	Hodgkin's disease	5 6	WCC[b] normal; progression, liver metastases and abdominal lymphomas, tracheo-oesophageal fistula, pneumonia, death 22 months after initial diagnosis
2	FM	fem.	21	B-symptoms: fever, night sweats, weight loss	Mediastinum	Malignant lymphoma	1 2 8 9	WCC normal; local progression, no distant metastasis or spread
3	WI	fem.	31	Thoracic pain (left), dry cough	Mediastinum	Malignant lymphoma	3a)	WCC normal; local progression, death due to respiratory insufficiency 11 months after initial diagnosis
4	LI	fem.	35	Painful right shoulder/thorax, moderate dyspnoea on exertion	Mediastinum	Malignant lymphoma	–	WCC normal; unknown, patient refused postoperative treatment
5	GS	fem.	35	Thoracic pain, dry cough, moderate dyspnoea on exertion	Mediastinum	Thymoma	3b) 4	WCC normal; local progression, lung involvement, distant metastases, death due to respiratory insufficiency 13 months after initial diagnosis
6	SS	fem.	43	Thoracical venectasia with cervical intumescence	Mediastinum	Malignant lymphoma	3c) 7	WCC normal; unknown
7	KM	male	22	Mediastinal enlargement, found in a routine x-ray	Mediastinum	Hodgkin's disease	–	WCC normal; local progression, no distant metastases
8	SG	male	29	Cervical swelling, facial and brachial oedema	(Para) thymic	Malignant lymphoma	3d)	WCC normal; progression, suspicion for liver and kidney metastases and abdominal lymph node involvement

[a] Preliminary diagnosis done on the basis of routine HE-sections for rapid notification; [b] white-cell count

(diluted 1:20) and a rabbit peroxidase-anti-peroxidase complex (diluted 1:100) were purchased from Dakopatts, Copenhagen, Denmark. Anti-human δ- and J-chain sera (diluted 1:80) were obtained from Nordic Immunology, Tilburg, Netherlands. Murine monoclonal antibodies to IgM and IgD (diluted 1:50), to the T_8-antigen (diluted 1:100), and to the leucocyte common antigen (Warnke et al. 1983) (diluted 1:40) were obtained from Dakopatts; the antibody to the T_4-antigen (Leu 3a; diluted 1:50) and antibodies to κ- and λ-chains (diluted 1:100) were purchased from Becton Dickinson, Mechelen, Belgium; the antibody to the common akute lymphoblastic leukaemia antigen (cALLa:J5; Ritz et al. 1981) (diluted 1:40) and the antibody to the T_{11}-antigen (Lyt 3; diluted 1:50) were obtained from NEN, Boston, MA, USA. The antibody to the B1-antigen (Stashenko et al. 1980; diluted 1:50) was purchased from Coulter Electronics, Hialeah, Florida, USA and the Ki67-antibody (Gerdes et al. 1983; diluted 1:50) from Dianova, Hamburg, FRG. The epitheliotropic monoclonal antibody HEA125 reacting broadly but absolutely restricted to normal and neoplastic epithelial cells (Momburg et al. 1985) was a kind gift from the producers G. Moldenhauer and F. Momburg (Institute for Immunology and Genetics, DKFZ, Heidelberg, FRG). A rabbit anti-mouse IgG and IgM antiserum (diluted 1:40) was supplied by Jackson Immuno Res. Lab. Inc., Avondale, USA.

"γ-Venin", i.e., pooled human immunoglobulin, was supplied by Behring, Frankfurt a.M., FRG. 3-amino-9-ethylcarbazole (AEC). N'N-dimethylformamide (DMF) were obtained from Sigma Chem. Co., St. Louis, MA USA and hydrogen peroxide, sodium acetate, and Pronase from Merck, Darmstadt, FRG.

Staining procedures. The bound antisera were detected via the standard three-step peroxidase-anti-peroxidase procedure. The monoclonal antibodies were detected by a four-step peroxidase-anti-peroxidase technique using a polyclonal rabbit-anti-mouse-Ig antiserum as a second step, followed by the linking antibody and the enzyme-anti-enzyme complex. All dilutions and washing steps were carried out with PBS pH 7.4, for dilutions containing 5 vol% γ-Venin to inhibit unsaturated tissue Fc receptors and to stabilize the antibody solutions. Incubations lasted for 30 min at room temperature. Using AEC/H_2O_2 (4 mg AEC dissolved in 500 µl DMF, added to 9.5 ml Na-acetate buffer (0.05M, pH 5.0), mixed with 5 µl H_2O_2 (30%) and filtered immediately prior to application), the peroxidase reaction resulted in an intense reddish-brown product. Incubation time was 5–15 min at room temperature. The sections were rinsed in tap water, counterstained with Harris' haematoxylin, and mounted with glycerol gelatine.

The detection of immunoglobulin components was carried out twice, using antisera on paraffin sections and monoclonal antibodies on frozen sections, in order to ensure reliable results. Positive controls were made in parallel using paraffin and frozen sections from tonsils, in order to ensure that a negative result on the tumour tissue was reliable and not due to the method. Negative controls performed by omitting the primary antibody or primary antiserum yielded negative results, except for the granulocytes in frozen sections, whose endogenous peroxidase was not destroyed.

Results

Clinics

The patients were young adults (19–43 years; mean 29.4) and predominantly female (6/8). First clinical symptoms consisted in thoracic pain, thorac venectasia; only in one case B symptoms were observed. The tumour localization was described as mediastinal in 6 and as (para-)thymic in 2 cases. At initial diagnosis there was documented involvement of the lungs in 3, of the pericardium in 2 and of cervical lymph nodes in 1 case; venous compression or invasion was observed in 3 patients. In retrospect it can be said the patients' blood group was the only laboratory variable that

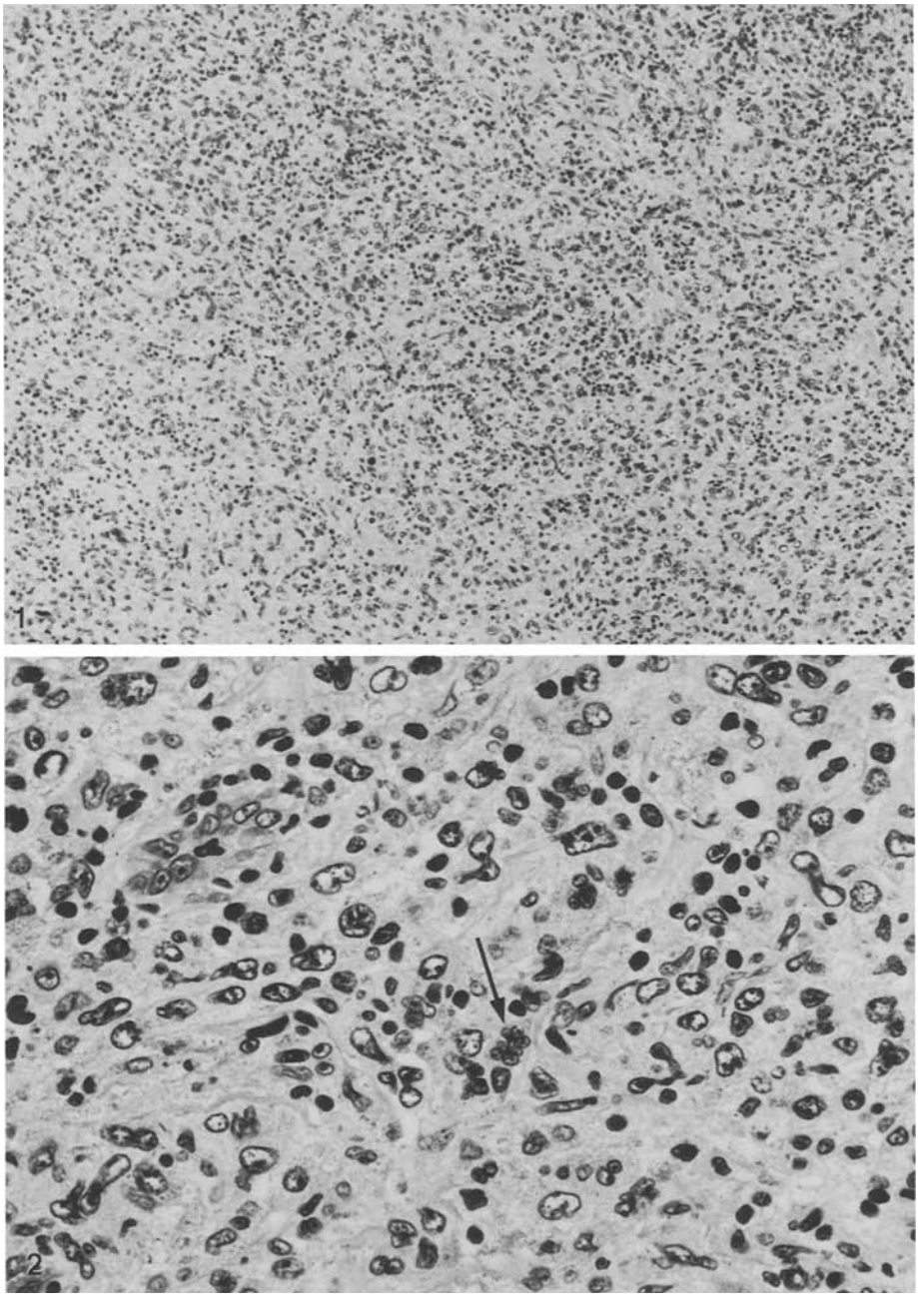

Fig. 1. Case 2: Low-power view: diffuse growth pattern; pleomorphic clear cells rich in cytoplasm, intermingled with small lymphocytes: thymoma-like aspect (HE, 116 ×)

Fig. 2. Case 2: Detail: tumour cells with abundant clear cytoplasm and irregular nuclei, club- to dumb-bell-shaped, sporadically multilobated (*arrow*!). Intermingled small lymphocytes, numerous apoptoses, plenty of fibres (HE, 460 ×)

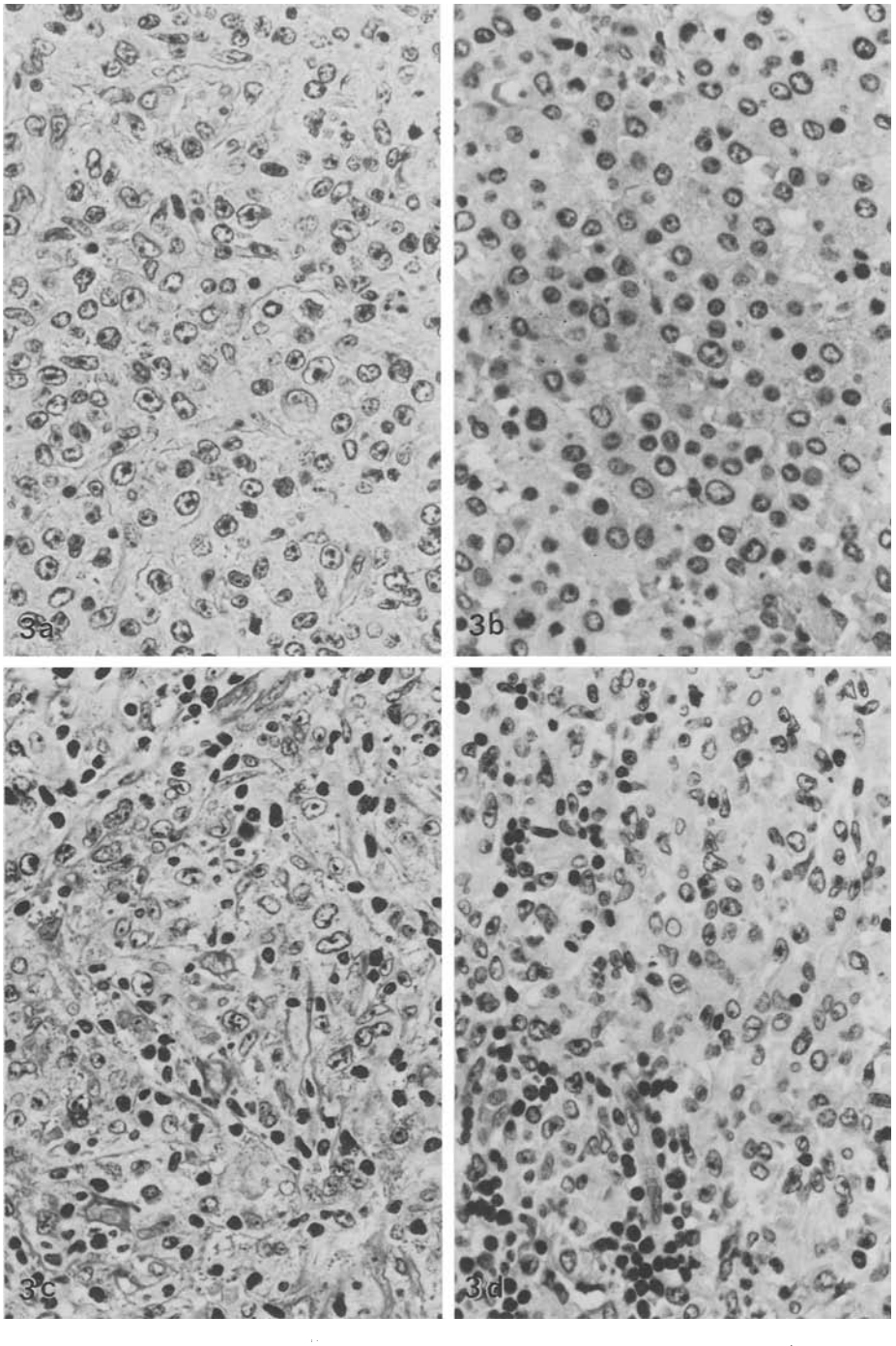

Fig. 3. Typical details of four cases, illustrating the morphological variability of the tumour cells (**a–e**: each HE, 320 ×). **a**) Case 3: large to medium sized tumour cells, clear cytoplasm; irregular, occasionally cleaved nuclei with prominent nucleoli, plenty of fibres. **b**) Case 5: medium-sized to relatively small and monomorphic tumour cells, broad and clear cytoplasm,

attracted attention: all of them were Rh+, 4 had O and 4 had A. The clinical course was characterized by therapeutic failure. Even though aggressive protocols against high grade lymphomas were applied in 6 cases, no remission was achieved, in contrast, progression was observed under therapy. Up to date three patients have died 11, 13 and 22 months, respectively, after initial diagnosis with extensive disease and large thoracic tumour masses causing respiratory insufficiency.

Histopathology

The tumours show a diffuse growth pattern (Fig. 1). The cells have clear and abundant cytoplasm in common, while they vary considerably in size. The nuclei are roundish to very irregular (Fig. 3), one tumour containing cells with multilobated nuclei (Fig. 2), a second containing giant cells with extremely pleomorphic, occasionally walnut-shaped nuclei (Fig. 6). In most of the cases the nuclear membrane is very distinct, the chromatin pattern is delicate. The nucleoli are predominantly small and more than one are found in only a small number of tumour cells. Generally speaking, in two cases the cells are best described as large, pleomorphic (Fig. 6), twice as mixed, large and medium sized, pleomorphic (Figs. 2 and 3a), twice as medium-sized, pleomorphic (Fig. 3c, d), once as medium-sized, monomorphic (case No. 4, not depicted), once as mixed, medium-sized to relatively small, monomorphic (Fig. 3b), even though the smallest tumour cells are twice as large as the diffusely infiltrating normal (T-)cells (Fig. 8) which are a regular constituent of the tumours, even though in varying quantities. Tumour necroses, mostly focal and small, but never confluent, are found in six cases. Mitoses are numerous and typical throughout. Even more frequent than mitoses are apoptoses (Fig. 6). The tumours are further characterized by an alveolar to micro-alveolar reticulin fibre scaffold (Fig. 4). In two cases a reticular fibrosis is a prominent feature (Fig. 3a, c) and in one patient pronounced sclerosis led regionally to a nodular-sclerosis pattern (Fig. 5). In conclusion, the histological picture of these tumours is not compatible with any of the known B-cell types listed in the Kiel classification. Routine histomorphology of these lymphomas resembles either pleomorphic peripheral T-cell lymphoma or thymoma, in one case even Hodgkin's disease.

Proliferation and DNA content

As suggested by the numerous mitoses, the proliferation rate is high in most cases. This is stressed by the finding that the proliferation-associated antigen Ki67 is detectable in 10 to 60% of the tumour cell nuclei (Fig. 9,

roundish nuclei, linear nuclear membrane and several nucleoli; only few small intermingled lymphocytes, numerous apoptoses. **c)** Case 6: medium-sized pleomorphic cells, oval, leptochromatic nuclei; reticular fibrosis. **d)** Case 8: medium-sized cells, pleomorphic oval to cleaved nuclei with spare chromatin and only sporadically enlarged nucleoli

Table 2. Proliferation and DNA content

Case no	Name	DNA Index	S-Phase	PI[a]	$\frac{G_2+M}{S}$	Ploidy	Ki67 %	Mitoses	Apoptoses
1	BM	1.00	3.8	5.9	0.55	2n	5–10	+++	++
2	FM	1.00 2.00	3.6	12.3	2.39	4n 8n	40	+	+++
3	WI	1.00	20.6	21.4	0.03	2n	60	++	++
4	LI	1.00	8.0	9.3	0.16	2n	30	+	++
5	GS	1.00	9.1	10.3	0.13	2n	50	++	+++
6	SS	0.80 1.62 1.00 2.00 1.25	10.7–14.0	21.0	0.50	2n	50	+	+++
7	KM	1.00	8.2	10.5	0.28	2n	40	++	++
8	SG	1.00	3.2	4.0	0.25	2n	10	++	++

[a] Proliferation index: $((S+G_2+M)\times 100/(G_{0_1}+S+G_2+M))$

Table 3. Immunophenotype of the tumour cell populations

Keratin	Negative	(8/8)
HEA 125[a]	Negative	(8/8)
LC	Positive	(8/8)
cALLa	Negative	(8/8)
B1	Positive	(8/8)
Ig's[b]	Negative	(8/8)
T_{11}, T_4, T_8	Negative	(8/8)

[a] Monoclonal antibody absolutely restricted to epithelial cells (vd. Methods)

[b] Immunoglobulin constituents including light and heavy chains and J-chain

Table 2). S-phase percentages as determined by cytophotometry are also considerably elevated, and in some cases to such extreme values as 20.6%, and so is the proliferation index (PI). The $(G_2+M)/S$-ratio is in seven cases <1 and as such highly abnormal and an indicator for the rapid run through the cell cycle. In connection with the high content of apoptoses these data illustrate the extremely elevated turnover within the tumour cell population. Six of the tumours are diploid, one is mixed, diploid and tetraploid, in another one 5 different aneuploid stem lines are detectable.

Immunohistology

The tumour cells of each case express LC-antigen but lack cALL-antigen. The B1 antigen is present in and on every tumour cell of each case (Fig. 7). Nevertheless, there are no Ig-constituents detectable, be it light or heavy chains, not even the J-chain, neither on the cell surface nor in the cytoplasm. Polytypic Ig is present only in mature plasma cells which, however, are very scarce. The small infiltrating lymphocytes are T cells (Fig. 8). The

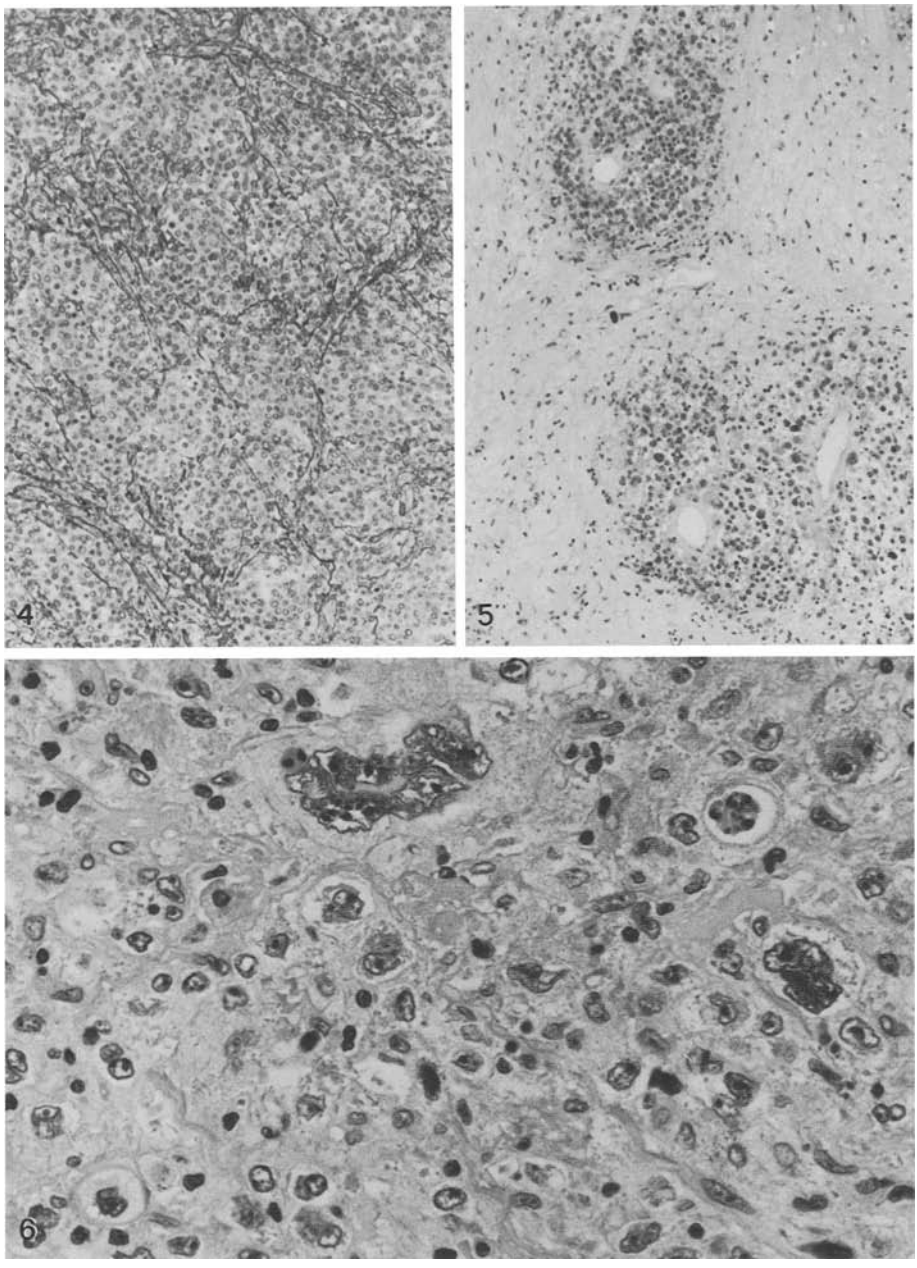

Fig. 4. Case 5: Silver stain reveals the alveolar to micro-alveolar reticulin scaffold typical of this tumour group (Pap 100 ×)

Fig. 5. Case 1: Aspect illustrating the pronounced tendency for fibrosclerosis detectable in six of the cases, in this special case mimicking nodular sclerosing Hodgkin's disease (vd. Fig. 6) (HE, 80 ×)

Fig. 6. Case 1: Similar to two other cases, this one contains a small number of multinucleated giant cells with multiple nucleoli. Some of them slightly resemble Sternberg-Reed cells leaving aside their walnutshaped nuclear contour. Note the numerous degenerating tumour cells (HE, 460 ×)

T_4/T_8 ratio is 2 to 3. There are no Ki67-positive small lymphocytes in any case. The tumour cells do not contain epithelial or T-cell markers.

Discussion

Clinical data

The tumours presented above are primary mediastinal non-Hodgkin's lymphomas. Taking into account the data up to now, the incidence of eight such cases within just two years of sampling seems extremely high for one institution only, but has to be related to the specialization of the hospital in thoracic surgery. Bosznyák et al. (1984) analyzed 644 mediastinal tumours and found that 27.3% were related to diseases of the mediastinal lymph nodes and 13% to thymic tumours. Among the first group Hodgkin's disease is predominant, being a tumour which in about 10% is limited to the mediastinal region on initial diagnosis and accounts for 50–60% of mediastinal malignant lymphomas (Otto 1984). Among the non-Hodgkin's lymphomas there are two lymphoblastic variants that tend to occur or arise in the mediastinum: Brittinger et al. (1984) report that in their extensive study comprising 1127 cases 60 (5.6%) were lymphoblastic lymphomas and from these 55% of the T-cell type and 41% of the unclassified type initially were located in the mediastinal and/or hilar region. Nevertheless, apart from the fact that none of our patients has become leukaemic, the sex ratio for lymphoblastic lymphomas (m:f=1.8:1) and their mean age of incidence (54 years, all data cited from Brittinger et al. 1984) clearly contrasts with our data. We found a pronounced female predominance and a mean age of 29 years indicating the peculiarity of the tumour group described above. The rapid deterioration of the patient's state despite aggressive anti-neoplastic therapy seems to be characteristic of this group and contrasts with the findings of Horning et al. (1984) who reported a better survival rate for their Ig^- large-cell lymphomas compared to the Ig^+ ones.

Histopathology

On morphological basis the tumours described cannot be classified according to the Kiel classification. There is a marked variation in the size of the tumour cells. Their main common characteristic is the clear and abundant cytoplasm. Some of our cases resemble cases 5, 6, and 7 of the series which Michandi et al. (1985) recently presented and which they called "'mixed lymphocytic/histiocytic' with large variations in size of abnormal cells"; their patients, however, were senile, had extensive disease, and the tumour cells contained surface or cytoplasmic single class Ig. Another case recently reported by Linch et al. (1985) is morphologically similar to our case 1 for its Hodgkin-like aspect. However, Linch's patient even though her cells were $B1^+/sIg^-$, was old, leukaemic and had generalized lymphadenopathy. The cells with multilobated nuclei of our case 2 are in fact B cells. Thus, as already stressed by Parfrey et al. (1985) and Weiss et al.

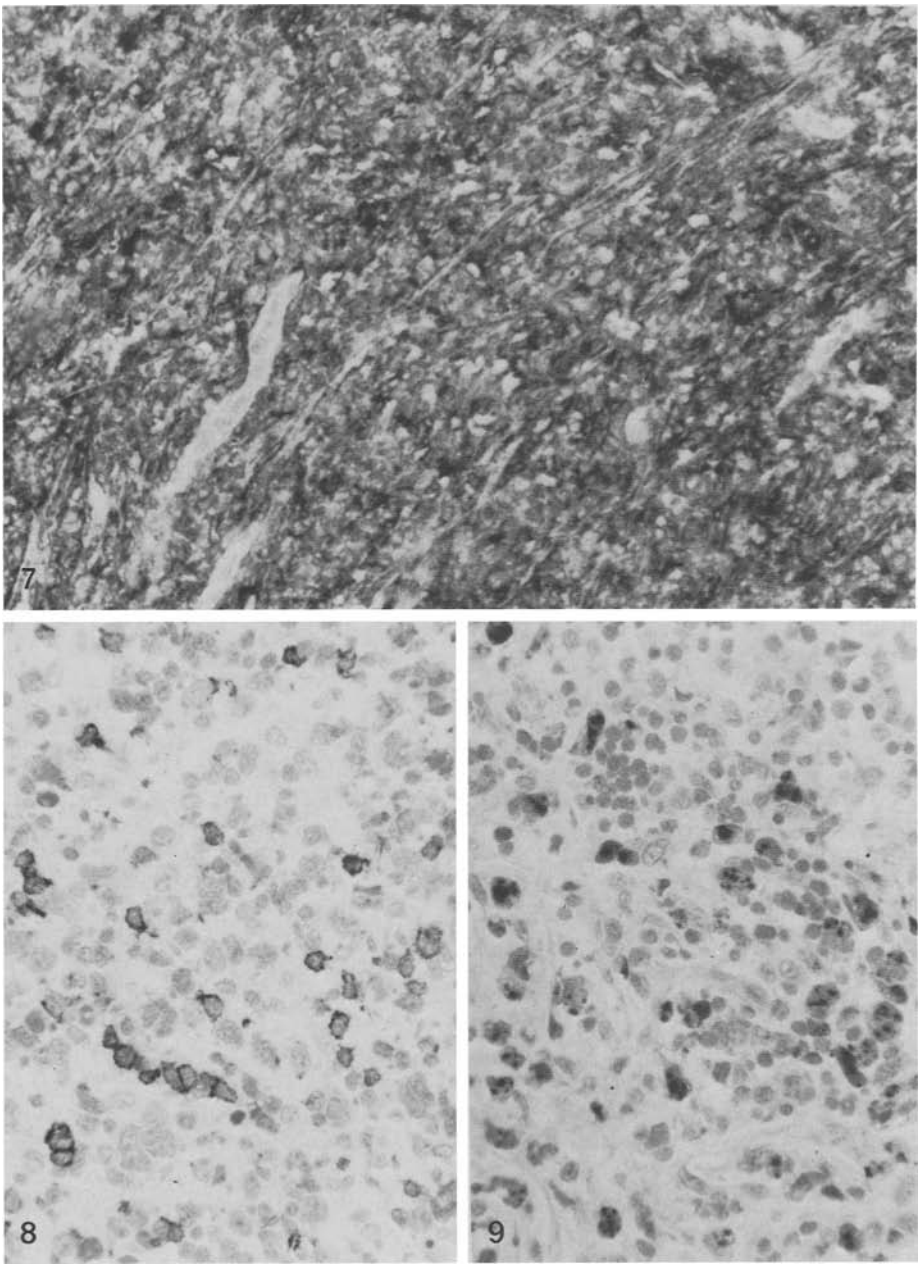

Fig. 7. Case 6: Immunohistochemical demonstration of the B1 antigen in a frozen section: every tumour cell is stained cytoplasmatically and on its surface, the result being an ill-defined, confluent immunoprecipitate. Note the non-reactive vessels and the negative connective tissue (AEC/H, 184 ×)

Fig. 8. Case 2: Paradigmatically demonstrating the T_8-antigen. The small lymphocytes are T cells, T-helper cells outnumbering the suppressor cells. The tumour cells are devoid of T-cell antigens (AEC/H, 250 ×)

Fig. 9. Case 2: Binding pattern of the Ki67-antibody detecting a proliferation-associated nuclear antigen: prevailingly nucleolar staining of a considerable number (in this case 40%) of the tumour cells, illustrating the proliferating activity in the tumour cell compartment while the lymphocytes are all Ki 67-negative (AEC/H, 250 ×)

(1985), this cytological variant cannot exclusively be attributed to the T-cell lineage as suggested by Pinkus et al. (1979).

Flow cytometry

Braylan et al. (1980) consider lymphomas with an S-phase share of more than 5% as highly malignant. Diamont et al. (1982) mark 2.2 to 11.1% of S-phases for high grade non-Hodgkin's lymphomas. Our tumour group contained percentages of cells in the S-phase ranging from 3.2 to 20.6%, according to Diamont et al. (1982), therefore 3 tumours belonged to the low, 4(3) to the intermediate and 1(2) to the high-grade malignancy group. Interestingly enough the three patients who have meanwhile died had S-phase shares of 3.8% (No 1), 9.1% (No 5), and 20.6% (No 3), thus covering the whole range. Another remarkable finding is the high number of diploid tumours (6) in our group. In contrast, Diamond et al. (op. cit.) report an overall percentage of 53 for aneuploidy in non-Hodgkin's lymphomas, of 71% for the intermediate and of 90% for the high-grade of malignancy. On the whole Diamond's data confirm those of Costa et al. (1981). To conclude, DNA-diploidy of the tumour stem line seems to be another characteristic of this lymphoma group.

Immunohistology

Our tumour group had the homogeneous immunophenotype LC^+, $cALLa^-$, $B1^+$, Ig^-, and T^-, illustrating its Ig-deficient B-cell nature. The value of LC for the differential diagnosis "lymphoma vs. carcinoma" is widely accepted (Warnke et al. 1983; Lauder et al. 1984; Gatter et al. 1985) except for plasmocytoma that seems to be devoid of LC (Salter et al. 1985). The aspect of a defective immunophenotype of malignant lymphomas has been studied on several occasions. Pallesen et al. (1983) report that 17% of their high-grade malignant lymphomas lacked both surface Ig and E-receptors; in another series Pallesen et al. (1984) studied 24 lymphomas devoid of cytoplasmic and surface Ig and E-receptors with lineage restricted monoclonal antibodies and found 18 B-cell lymphomas, 14 among them reacting with the B1-antibody. The B1 antigen as lineage marker served Horning et al. (1984) and Freedman et al. (1985) to define the B-cell nature of a subgroup of their Ig^- large cell lymphomas. Thus, and this has been confirmed by our own results, the B1-antigen is a reliable B-cell marker and apparently more stable in the course of malignant transformation than Ig expression. Non-neoplastic fetal B cells, early adult B cells and germinal centre cells express cALLa on their surface (Hsu and Jaffe 1984; Delia et al. 1985), and so do the lymphomas derived from them (Bernhard et al. 1982; Swerdlow et al. 1983; Cossman et al. 1984). Even the follicle centre cell lymphomas deficient in Ig published by Gregg et al. (1984) expressed the B1- and the cALLa-antigen. It therefore seems unlikely that our tumour group is closely related to the germinal centre cell lymphoma group. However, the normal counterpart of the cells giving rise to the mediastinal clear cell lymphoma of B-cell type remains enigmatic at present.

To conclude, these eight non leukaemic clear cell tumours of the mediastinum characterized by their occurrence in young, predominantly female adults, their defective B-cell immunophenotype, their rapid cellular turnover and bad clinical course seem to belong to a tumour type not described so far and probably represent a novel "entity" among B-cell neoplasias.

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